A novel immobilization method of an active protein via in vitro
N-terminus specific incorporation system of non-natural amino acids

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ABSTRACT
Recently, we succeeded in incorporating a biotin tag into an active protein, but only at the N-terminal site, in the presence of an Escherichia coli initiator tRNA^Met aminoacylated with methionine biotinylated at the α-amino group. The biotinylated protein was immobilized on a streptavidin-matrix. We have tried to increase the biotin labeling efficiency by decreasing concentrations of non-biotinylated tRNA^Met in the translation system.

INTRODUCTION
Biotinylation of a protein in general involves a chemical modification of a translated protein. However, with this methodology, biotinylation at a specific position remains difficult. We have previously reported that a biotin tag could be incorporated into a green fluorescent protein (GFP), but only at the N-terminal site, in the presence of an E. coli initiator tRNA^Met aminoacylated with methionine biotinylated at the α-amino group. We also confirmed that the N-terminal biotinylated protein retained its native activity, and was immobilized onto the solid support of streptavidin. However, an intrinsic problem with this system is that the biotin labeling efficiency is rather low; i.e., it is saturated up to ca. 10-20%. Here we have attempted to improve the labeling efficiency and in doing so obtained insights of some new aspects.

Figure 1. Syntheses of chemically modified tRNA^Met.

hydrolysis

\[ \text{aqueous buffer (pH 8.5)} \]

\[ \text{BODIPY FL-SSE} \]

\[ \text{Biotin-SSE} \]

\[ \text{tRNA^fmet} \]
RESULTS AND DISCUSSION
The synthesis of chemically modified tRNA
\(^{\text{mnt}}\) (4) is shown in Figure 1. When Met-tRNA
\(^{\text{mnt}}\) (2) was reacted with the succinimidyl ester (3) to obtain the target molecule (4), the extent of the Met-tRNA
\(^{\text{mnt}}\) derivation was ca. 50% as determined by HPLC analysis (Figure 2b). As the coupling reaction did not reach 100%, non-biotinylated Met-tRNA
\(^{\text{mnt}}\) (2) still remained in the system. Deaminoacylated byproduct (1) was also obtained by the unfavorable hydrolysis of 2 under the reaction conditions. In earlier reports\(^1\), the tRNAs (1, 2, and 4) were used in the subsequent translation experiments without further purification. We postulated that in the presence of certain amounts of non-functionalized tRNAs (1, 2), the AUG initiation codon would be preferentially recognized by the natural, non-functionalized tRNA. Thus, the separation of chemically modified tRNA
\(^{\text{mnt}}\) (4) was carried out by hydrophobic interaction chromatography\(^3\), followed by G-25 filtration chromatography and the ethanol precipitation. As shown in Figure 2c, non-functionalized tRNAs (1, 2) were efficiently removed from the system; the amount of non-functionalized tRNAs being less than one fifth of 4.

The labeling efficiency, by using the purified tRNA
\(^{\text{mnt}}\) (4), was estimated from SDS-PAGE densitometric analysis. It was, consequentially, the same as the previous results\(^1\) (data not shown). The AUG initiation codon was preferentially recognized by the non-functionalized tRNAs (1, 2), even the amount of functionalized tRNA
\(^{\text{mnt}}\) (4) was much larger than those of the non-functionalized ones (1, 2). These results indicate that recognition of the E. coli initiator tRNA
\(^{\text{mnt}}\) aminoacylated with a nonnatural amino acid was strongly restricted by an E. coli ribosome initiating the in vitro translation.

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REFERENCES AND NOTES